AMENDMENTS TO THE SPECIFICATION

Please replace paragraph [0046] with the following replacement paragraph.

[0046] For the purposes of obtaining terminal embryo development, manipulation of the developmental stage of embryos by affecting the water potential of the embryogenic tissue, either by separating the somatic embryos from a liquid medium by a porous support, or by introducing a gelling agent (e.g. gellan gum) into the growth medium in larger than normal quantities, is taught in U.S. Patent 6,200,809. In the present invention, we employ similar means to affect the developmental stage of the embryos during selection, not to favor terminal embryo development. As taught in U.S. Patents 5,506,136 and 5,856,191, primary initiation of *Pinus* embryogenic cultures is favored not only by ABA in the medium at similar concentrations, but also by manipulation of the matrix potential by use of a GELTRITE® gelling agent concentration lower than that commonly taught in plant tissue culture, i.e. between 0.5% and 2 % GELRITE® gelling agent in DCR nutrient media, wherein previous methods had used 2% GELRITE® gelling agent in media of similar salt composition, and 2% GELRITE® gelling agent is also commonly used in maintenance and selection media of similar salt composition. Accordingly, we hypothesized that manipulation of the matrix potential in transformation, recovery and selection media might enhance initiation of secondary somatic embryogenesis in transformed precotyledonary embryos, particularly in some lines in which precocious embryo development is observed in the absence of such manipulation.

Please replace paragraph [0057] with the following replacement paragraph.

[0057] After megagametophyte explants were placed in culture, the perimeter of the dish was sealed with two wraps of NESCOFILM® sealing film (commercially available from Karlan Company). The dishes were incubated in the dark at a constant temperature of 23° ± 2°C. After about 7 to 21 days, embryogenic tissue extruded from the micropyle of the megagametophyte

explants. At six weeks following the placement of the explant on initiation media, tissue masses that had extruded and were proliferating from individual explants were isolated to individual petri plates on maintenance medium DCR_2 or $WV5_2$ and assigned line numbers. After one to three months of culture on maintenance medium, the tissue cultures were cryopreserved.

Please replace paragraph [0059] with the following replacement paragraph.

[0059] Frozen cultures were retrieved when desired by removing individual vials from the cryobiological storage vessel and placed in 42° ± 2°C water to rapidly thaw the frozen cell suspensions. The thawed cell suspensions were aseptically poured from the cryovial onto a sterile 35 μm pore size polyester membrane support placed over sterile filter paper (Whatman® no. 2 filter paper, Whatman International Ltd.) for a few minutes to allow the DMSO cryoprotectant solution to diffuse away from the embryogenic tissue into the paper. The embryogenic tissue on the polyester support membrane was then transferred to DCR_2 maintenance medium and incubated at 23° ± 2°C in the dark for 24 hours to allow additional DMSO to diffuse away from the tissue into the medium. The polyester support bearing the embryogenic tissue was then removed from the medium and transferred to fresh DCR_2maintenance medium, and thereafter, every 14-21 days to a fresh plate until the amount of cells per plate reached about 1 g. The culture environment during post-cryopreservation recovery and growth was 23° ± 2°C in the dark. Those skilled in the art will recognize that many different cryopreservation and recovery procedures would be suitable for use with this method and the detail in this example may not be construed to limit the application of the method.

Please replace paragraph [0061] with the following replacement paragraph.

[0061] To prepare for gene transfer, a sterile fabric support (in this example PECAP® fabric support, commercially available from SEFAR Inc.) was placed in a sterile Buchner funnel and one

to five milliliters of embryogenic suspension was pipetted onto the fabric support such that the embryogenic tissue was evenly distributed over the surface. The liquid medium was suctioned from the tissues using a mild vacuum. The fabric support with embryogenic tissue was removed from the Buchner funnel and placed on a GELRITE® gelling agent solidified DCR_3 preparation medium (Table 2) in 100 X 25 mm plastic petri dishes. Dishes were incubated in a dark growth chamber at 23° ± 2°C for about 24-48 hours.

Please replace paragraph [0063] with the following replacement paragraph.

[0063] The petri dishes with the fabric support and embryonic tissues were then placed into the interior of the PDS 1000/He BIOLISTICS® device and vacuum applied to a level of 28 inches Hg. The gold particles carrying the DNA were accelerated toward the embryogenic tissue following a helium build-up and bursting regulated by a 1550 psi rupture disk. In the PDS-1000/He BIOLISTICS® device the gap between the rupture disk and the macrocarrier (gap distance) was five mm and the macrocarrier travel distance was 13 mm. Following DNA transfer the petri dishes containing the fabric support and tissues were incubated in a dark growth chamber at 23° ± 2°C for about 24 hours. The tissues and fabric support were transferred to semi-solid maintenance medium, DCR_1 (Table 2) to recover from carrier particle bombardment and incubated in a dark growth chamber at 23° ± 2°C for a period of about seven days. The tissues and fabric support were transferred to a selection medium, semi-solid maintenance medium DCR_1 containing a level of selection agent inhibitory to the growth of non-transformed cells. In this and subsequent examples the selection agent used was GENETICIN® antibiotic at 15-30 mg/l. The plates were incubated in a dark growth chamber at 23° ± 2°C for about six to twelve weeks with the fabric supports containing the tissues being transferred to the same fresh culture medium every 2-3 weeks.

Please replace paragraph [0074] with the following replacement paragraph.

[0074] After a total of ten weeks of selection, the plates were examined for sublines growing in the presence of the GENETICIN® antibiotic selection agent, and cells from these sublines were observed for staining indicating the presence of the *uidA* transgene. The cells were also checked for the presence of sequences by PCR amplification using primers specific for both the *uidA* and *nptII* transgenes, techniques well known to those skilled in the art of plant transformation.

Please replace paragraph [0088] with the following replacement paragraph.

[0088] Following co-cultivation, cells were re-suspended into fresh DCR_4 liquid wash medium (Table 2) containing eradicants such as 200-400 mg/L TIMENTIN® antibiotic. The DCR_4 liquid wash medium was contained in sterile "baby food" jars with MAGENTA® aerated lids, conventional beakers, or multi-well plates. Resuspension was initiated by grasping the membrane support bearing the infected cells, using forceps, and rolling or folding it so that it could be taken up and placed into the liquid in the wash container. The liquid was then agitated to get the cells into suspension, and the membrane support was scraped with sterile forceps if cells appeared to be adhering to it. Once the cells were in suspension, the membrane was removed with sterile forceps.

Please replace paragraph [0090] with the following replacement paragraph.

[0090] Supports bearing approximately 0.1 g of embryogenic tissue were divided onto recovery media (having the same formulation as the maintenance medium except for the addition of 400 mg/L TIMENTIN® antibiotic) either containing or lacking ABA. Following a one-week recovery period during which the cells were observed for resurgence of *Agrobacterium*, the polyester support membranes bearing the pine somatic embryogenic tissue were divided onto DCR selection

media either containing or lacking ABA. Concentrations of ABA used in all these media were 0, 10, and 30 mg/l.